

SEQUENCE MATCHING, SIMPLE SEARCHING

PGA Course in Bioinformatics
Tools for Comparative Analysis

July 15, 2002

Outline

Sequence alignment algorithms

- Rigorous Optimality:Needleman-Wunsch and Smith-Waterman
- Rapid, heuristic algorithms
 - BLAST
 - FASTA
 - and their relatives

Databases and Search Tools

MAJOR SITES WE WILL USE

☞ <http://www.ncbi.nlm.nih.gov/>

☞ <http://workbench.sdsc.edu>

Needleman Wunsch Algorithm

- ☞ Global alignment:: every residue of the two sequences has to participate
- ☞ Guaranteed to calculate an Optimal similarity score
- ☞ Begin at the beginning of each sequence and go to the end.
- ☞ Cannot detect domains

Smith-Waterman Algorithm

- ☞ Optimal Local Alignment
- ☞ Guaranteed to find all significant matches to a given query
- ☞ Takes the query sequence versus every sequence in the database
- ☞ Can be used with arbitrary scoring systems
- ☞ **COMPUTATIONALLY EXPENSIVE!!!**

Scoring Matrices

- ☞ Relatively simple for DNA-gap penalties or mismatches-can be made to look at Pu/Py
- ☞ Protein matches look also at similarity (leu/ileu)

Protein Scoring Matrices

- Chemical similarity: 210 pairs of aa
- Nearness in Genetic Code
- Chemical similarity, e.g.,
hydrophobicity
- Observed Substitution Schemes

Observed AA Substitution Matrices

- PAM
- BLOSUM

PAM: Point Accepted Mutation

- ☞ DAYHOFF et al.
- ☞ Observed residue replacement in related proteins
- ☞ GLOBAL alignment, closely related
- ☞ A model of molecular evolution
 - 1 PAM = average change in 1% of all amino acid possibilities(1% divergence)
- ☞ Other PAM matrices extrapolated from PAM1.

PAM continued

- ☞ TIME is NOT correlated with PAM
- ☞ Number of the matrix refers to evolutionary distance

Means different families of proteins evolve at different rates

PAM250

Table 3: The PAM250 matrix – an example of a matrix derived from observed substitutions

A	B	C	D	E	F	G	H	I	K	L	M	N	P	Q	R	S	T	V	W	Y	Z
A	2	-1	0	3	-4	1	-1	-1	-2	0	1	0	-1	-1	1	-1	-4	-1	0		
B	0	2	-1	2	3	-5	0	1	-1	-2	2	-1	1	-1	4	0	-1	-5	-1	2	
C	-2	-4	1	-5	-4	0	-1	-2	-1	-6	-5	-4	-1	-1	0	-2	-1	-8	-5		
D	0	8	3	-6	4	3	-4	1	-1	0	-4	0	2	-1	2	-1	0	-1	-7	-4	
E	3	8	2	4	-5	8	1	-2	0	-3	-2	1	-1	2	-1	0	0	-2	-7	-8	
F	-4	-6	-4	-4	-5	9	-1	1	-5	3	0	-4	-5	-1	0	-2	-1	0	-7	-5	
G	1	0	-1	1	0	-1	-2	-1	-2	0	0	-1	-1	1	0	-1	-5	1			
H	-1	1	-1	1	0	-2	1	4	-4	0	-4	-2	1	0	1	2	-1	-2	-1	0	
I	-1	-2	-1	-2	-1	-1	-2	3	-2	3	2	-2	-2	-2	-1	0	0	-1	-1	-1	
K	0	1	-1	0	0	-1	0	-2	5	-1	0	1	-1	1	0	0	-2	-3	-4	0	
L	-1	-2	-1	-2	-1	-2	-2	0	-1	4	-1	-2	-3	-1	-2	1	-2	1	-1	0	
M	-1	-1	-2	-2	0	-3	-2	2	0	4	-2	-3	-1	4	-1	0	1	3	-4	-2	
N	0	3	-4	0	1	-4	0	-2	-3	-2	0	1	1	0	1	0	-2	-1	0		
P	-1	1	0	-1	-1	0	-2	-1	-2	-1	0	-1	4	0	1	0	-1	-4	0		
Q	0	0	1	-8	3	2	-1	0	-3	1	-1	0	0	1	-1	0	-1	0	-8	0	
R	-2	-1	-4	-1	-1	-2	3	-2	3	-1	0	0	1	1	-1	-2	2	-4	0		
S	1	1	0	0	0	-3	1	-1	1	0	-1	-2	1	1	-1	0	2	1	-1	0	
T	1	1	0	-2	0	0	-1	0	0	-2	1	0	-1	-1	1	0	0	-2	-1		
V	0	-2	-2	-2	0	-1	-2	4	-2	2	3	-2	-1	-2	1	0	4	-6	-2		
W	-4	0	0	-7	0	-7	0	-3	-2	0	-6	-4	0	0	2	-2	-6	17	0		
Y	-3	-2	0	-4	-6	7	-5	0	-1	-4	-1	-2	-1	-1	-4	-6	-2	0	20	-4	
Z	0	3	-8	3	0	-1	2	-2	0	-2	1	0	3	0	0	-1	0	-6	3		

The non-standard scores used in the matrix differ by 0.05. T-value = 40 = 270.

Blosum62

	A	C	D	E	F	G	H	→
A	4	0	-2	-1	-2	0	-2	
C	0	9	-3	-4	-2	-3	-3	
D	-2	-3	6	2	-3	-1	-1	
E	-1	-4	2	5	-3	-2	0	
F	-2	-2	-3	-3	6	-3		
G	0	-3	-1	-2	-3			
H	-2	-3	-1					
								BLOSUM 62

BLOSUM

- ☞ Block Substitution Matrix
- ☞ Henikoff and Henikoff, PNAS, 1992
- ☞ Number following indicates per cent identity within set, BLOSUM62=62% id
- ☞ Finds short, highly similar sequences (no gaps)

BLOSUM

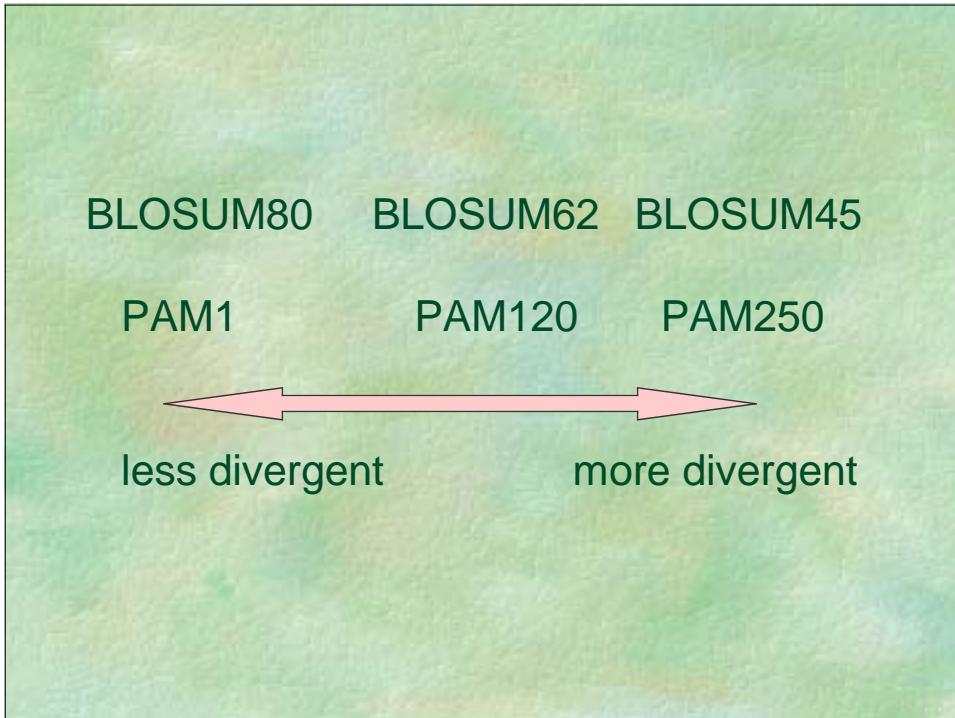
- ☞ Matrices are directly calculated, based on observed alignments
- ☞ Greater numbers are lesser distances
- ☞ Usually best for local similarity searches
- ☞ BLOSUM62= DEFAULT FOR BLAST. If a distant relative, think about another matrix.

BLOSUM SCORING RULES

- ☞ Zero score means the frequencies of the pair in the database is that expected by chance
- ☞ A positive score means more frequent than chance
- ☞ Negative score means the pair is found less frequently than chance.

BLAST-Basic Local Alignment Sequence Tool

- ☞ Objective: find all local regions of similarity distinguishable from random
- ☞ Only local alignments permitted,
- ☞ Gaps permitted in version 2
- ☞ Statistically sound (Karlin and Altschul), but no guarantee of optimality



BLAST: Three Step Algorithm

- » Compile a list of high scoring words of length w ($w=4$ for proteins, 12 for nucleic acids)
- » Scan for word hits of score greater than threshold, T
- » Extend word hit in both directions to find High Scoring Pairs with scores greater than S

Other BLAST Programs

- ☞ BLASTN: nucleic acid query to NA database
- ☞ BLASTP: Protein query to Protein database
- ☞ BLASTX: Translated nucleic acid query to Protein database
- ☞ TBLASTN: Protein query against (translated) nucleic acid database
- ☞ TBLASTX: Translated nucleic acid against translated nucleic acid database

OTHER BLAST VARIATIONS

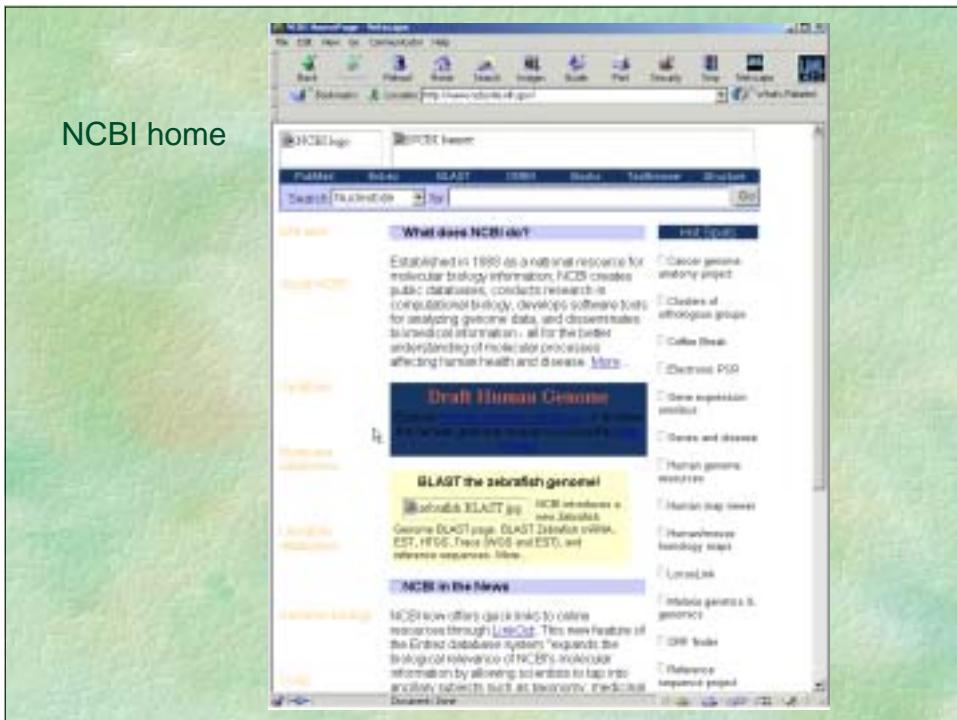
- ☞ Gapped BLAST (BLAST 2.0) -extend words from no-gap to gap, generate gapped alignments
- ☞ PSI-BLAST- Position Specific Iterated BLAST-use gapped BLAST, generate a Profile from multiple iterations used instead of the input and Distance Matrix

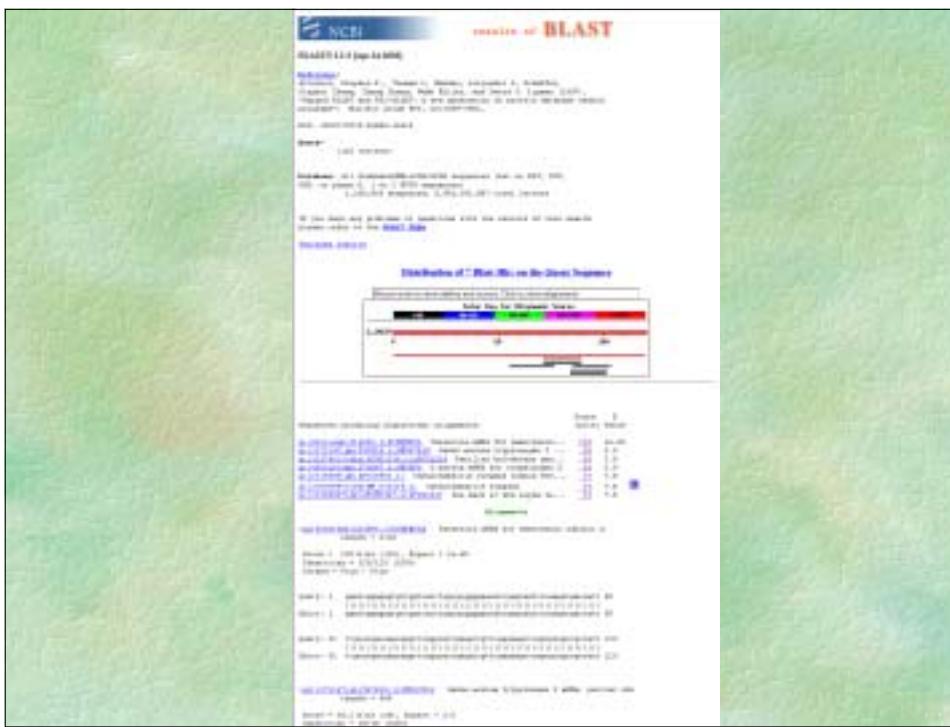
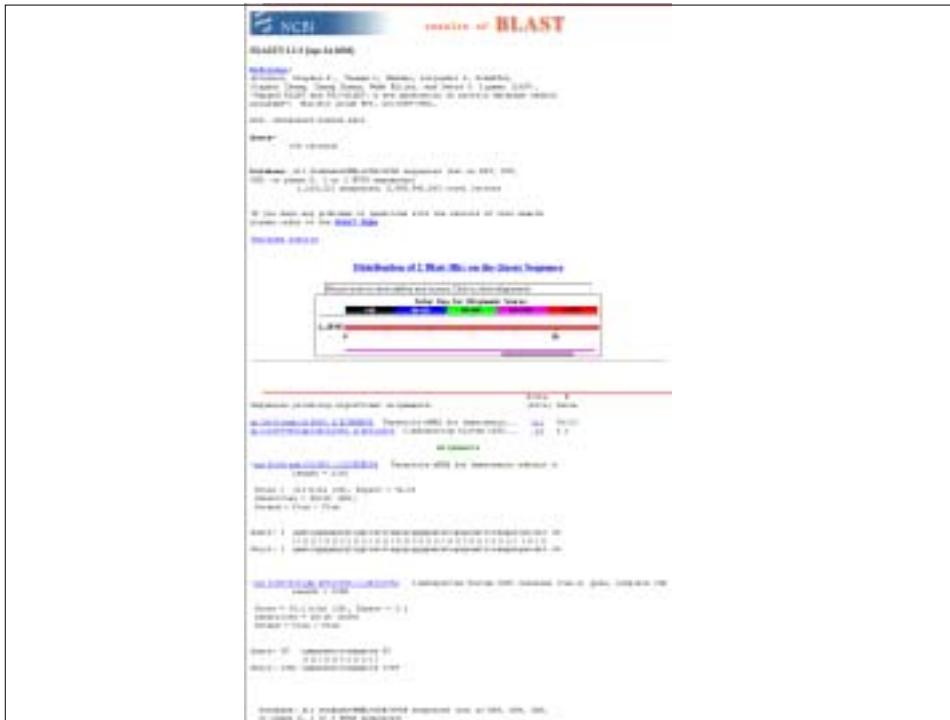
Limitations to BLAST

- ☞ Needs islands of strong homology
- ☞ Limits on the combination of scoring and penalty values
- ☞ The variants (blastx, tblastn, tblastx) use 6-frame translation-miss sequences with frameshifts)
- ☞ Finds and reports ONLY local alignments

A WALK THROUGH BLAST

NCBI home

A screenshot of the NCBI BLAST search results page. The top navigation bar includes links for NCBI Home, Picture, Tutorial, and Previous results for an ID. Below the navigation bar, a message says "Your request has been successfully submitted and put into the Blast Queue". A blue checkmark icon is placed next to the message. Below this, it says "Query = (60 letters)". A text input field contains the text "The request ID is 1025631000-08133-31100". Below the input field is a "Format" button with the value "FORMAT=HTML". A note below the button says "This results are estimated to be ready in 30 minutes but may be done sooner." It also says "Please press 'FORMAT=' when you wish to download your results. You may change the formatting options for your result via the form below and press 'FORMAT=' again. You may also request results of a different search by entering any other valid request ID to see other recent jobs." A large red rectangular box highlights the "Format" section.





BLAST RULES OF THUMB

- ➲ For short amino acid sequences (20-40), 50% identity happens by chance
- ➲ If A and B are homologous, and B and C are homologous, then A and C are, even if you can't see it.
- ➲ You can get similarity in the absence of homology for low complexity, transmembrane and coiled-coil regions. These have to be eliminated by you.

BLAST Significance

- ☞ If you change scoring systems, you can still compare search results if you normalize the score.

$S' = (\lambda S - \ln K) / \ln 2$. Lambda and K are associated with the scoring system.

S' , with a given E, is significant if it is greater than $\log N/E$, N the size of the search space.

FASTA: FAST Alignment

☞ <http://alpha10.bioch.virginia.edu/fasta/>

☞ <http://www2.ebi.ac.uk/fasta3>

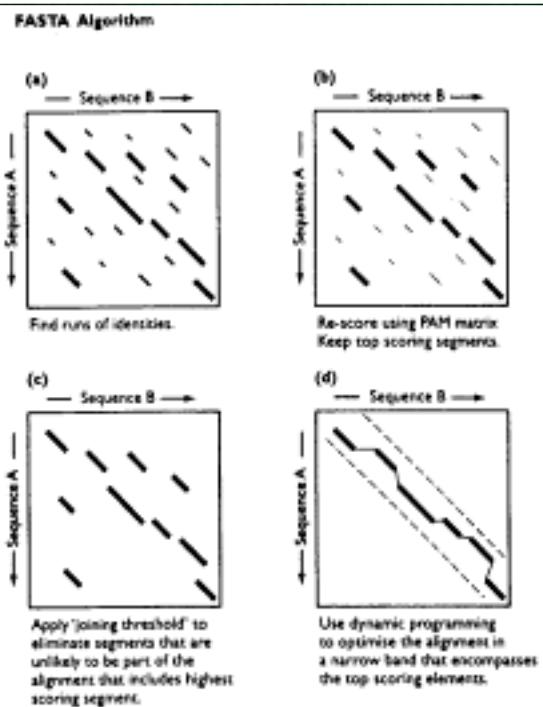
☞ <http://workbench.sdsc.edu>

☞ Rapid Global alignment

☞ Not a strong mathematical basis

FASTA: WHY USE IT?

- Allow alignments to shift frames

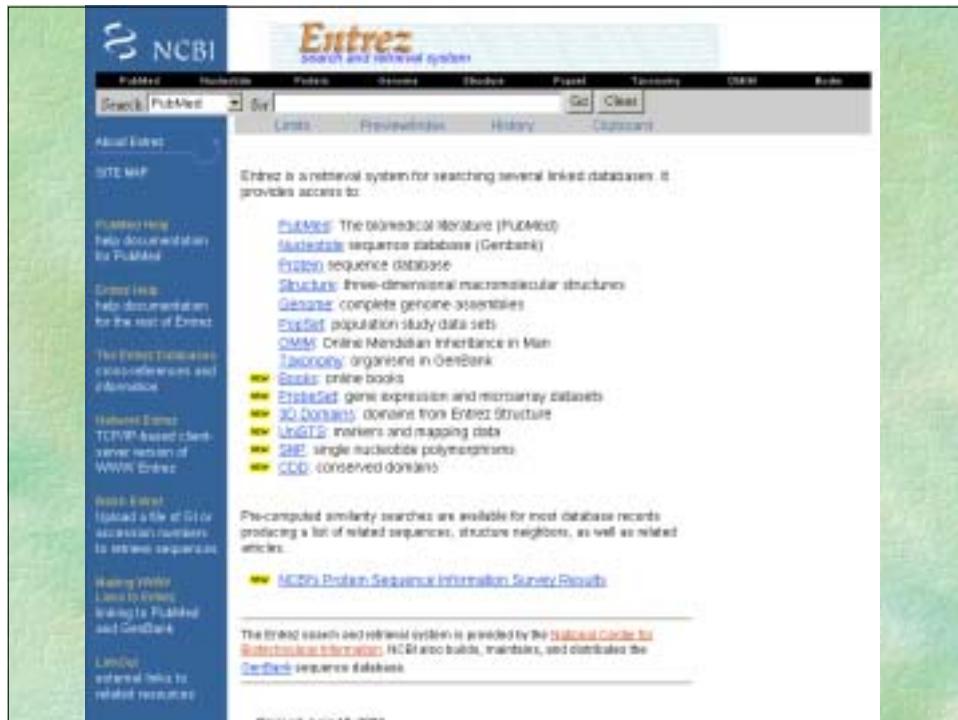


LALIGN

- ☞ Essentially a FASTA derivative for local alignments
- ☞ Compares two proteins to identify regions of similarity
- ☞ Will report several sequence alignments within a given sequence
- ☞ Works for internal repeats that are missed by FASTA because of gaps.

SITES for LALIGN

- ☞ <http://fasta.bioch.virginia.edu/fasta/lalign.htm>
- ☞ <http://xylian.igh.cnrs.fr/bin/lalign-guess.cgi>
- ☞ <http://biowb.sdsc.edu> (registration necessary but painless)
- ☞ **PALIGN**
<http://fasta.bioch.virginia.edu/fasta/palign.htm>
(plots a graph of the areas of alignment)



ENTREZ: Linked Databases

<http://www.ncbi.nlm.nih.gov/Entrez/>

- ☞ Concept of Neighbor-usually BLAST relationship
- ☞ Precomputed=Fast
- ☞ Related sequence, structure neighbors, related articles

EST DATABASES:Quality issues

SEQUENCE QUALITY

- calculated error less than 1% (Phred-20) is the rule
- frameshifts and stops common
- Rules are usually observed by exception
- There are lots of exceptions in the public data
- Many 3' UTRs

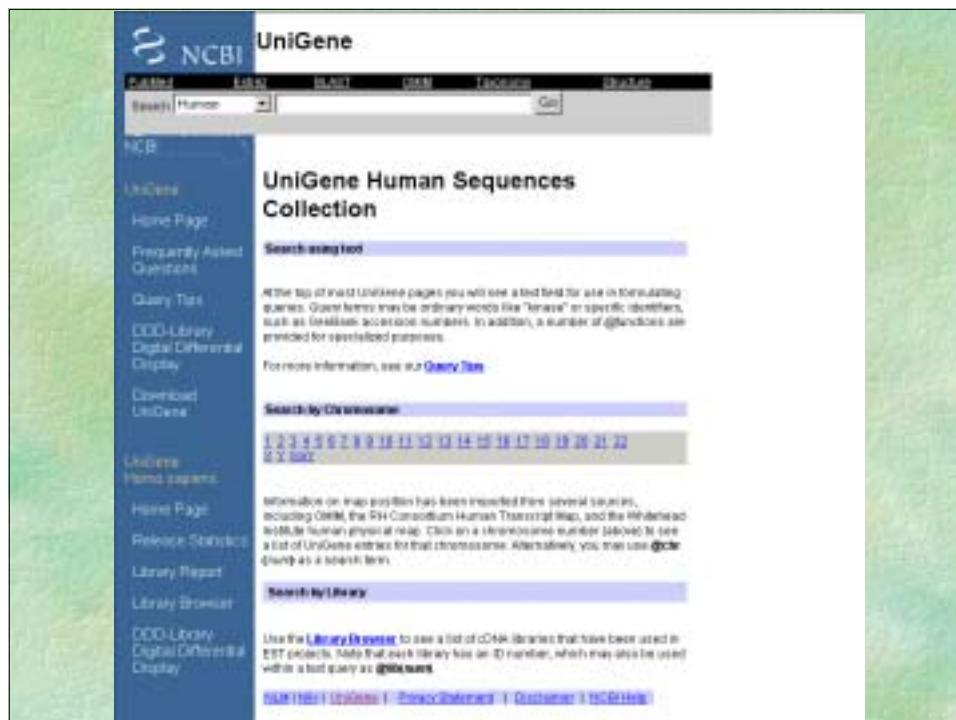
EST Databases: Quality #2

CLONE QUALITY

- Over-representation
- Tissue specificity
- Developmental stage specificity
- Unprocessed mRNA clones
- Chimeras
- Contamination

EST Cluster Databases

- » STACK-at SANBI <http://sanbi.ac.za>
- » TIGR-animals, plants, other
<http://www.tigr.org/tdb/tgi.shtml>
- » Unigene-NCBI
 - Human, mouse, rat, cow, zebrafish
 - mRNAs
 - predicted mRNAs



The screenshot shows the UniGene Human Sequences Collection homepage. At the top, there's a search bar with 'Species: Human' and a 'Go' button. To the left is a sidebar with links like 'UniGene Home Page', 'Frequently Asked Questions', 'Query Tools', 'DDG-Library Digital Differential Display', 'Download UniGene', 'UniGene Home Page', 'Help', 'Reference Statistics', 'Library Report', 'Library Browser', and 'DDG-Library Digital Differential Display'. The main content area has three search sections: 'Search by gene name', 'Search by chromosome' (listing chromosomes 1-22, X, Y, and GENE), and 'Search by library'. Below these is a note about Ensembl and a footer with links to 'UniGene Home Page', 'Help', 'Reference Statistics', 'Library Report', 'Library Browser', and 'DDG-Library Digital Differential Display'.

UNIGENE

➲ A LIST OF LISTS

- The cluster and known EST, mRNA pieces
- Additional annotation-gene name, etc.
- Distributed as a subset of dbEST

NOT included in the BLAST searchable DB
at NCBI

Caveats on Clusters

- ➲ Not stable
- ➲ Can go to complete cDNAs as available

LOCUSLINK

(<http://www.ncbi.nlm.nih.gov/LocusLink>)

- ➲ A useful, searchable compendium of loci across human, mouse, rat, Drosophila and zebrafish
- ➲ Linked for PubMed, OMIM, RefSeq, Homologene data, Unigene, and Variation Data

The screenshot shows the LocusLink search results for the gene RNF10. At the top, there's a navigation bar with links for Home, Search, Help, and Log In. Below it is a search bar with the query 'RNF10' and a 'Search' button. The main content area has several sections:

- Summary:** Provides basic information about the gene, including its ID (RNF10), name (RNF10), and description (RNF10, also known as RNF10). It also lists other names: RNF10, RNF10, RNF10, and RNF10.
- Gene Structure:** Shows the genomic structure of the RNF10 gene, including exons (red) and introns (blue).
- Protein Product:** Details the protein product, including its ID (NP_001002), name (RNF10), and description (RNF10, also known as RNF10).
- Gene Ontology:** Lists biological processes, cellular components, and molecular functions associated with the gene.
- Protein Domains:** Lists various domains found in the protein product.
- Conservation:** Shows conservation scores across different species.
- Gene Expression:** Displays gene expression data from various sources.
- Protein Interaction:** Lists known interactions for the protein product.
- Gene Network:** Shows interactions between RNF10 and other genes.
- Gene Environment:** Lists nearby genes and their descriptions.
- Gene History:** Shows the history of the gene entry.
- Gene References:** Lists publications related to the gene.
- Gene Aliases:** Lists alternative names for the gene.
- Gene Symbols:** Lists other symbols for the gene.
- Gene ID:** Shows the primary gene ID (RNF10).

NCBI LocusLink

Search: LocusLink Display: Summary Organism: All

Query: TSHB

View Loc. Save Loc.

LocusLink Home Help

Basic Search			
GeneID	Org. Symbol	Description	Protein Link
1916	HLA-B	HLA-B-associated transcript 2	Op2.3
		Aliases:	G13D63L, G6511R, I42100, NM_004625, NM_004626, NM_004628, NM_004629, NM_08868 AF29156, AP00030, M03143, M03148, Z13921, AY058239, SC3338
		Molecules:	HP_084628, HP_342413, HP_084629, HP_342413, AAE1896, BAE8351, AAA22386, AAA22386, CAA37744, AAA22385
		Protein:	13
		RefSeq:	Q2_Bal2_Bal_<M 2_Wig12 C1_995521E 3118396812A 3118396812B
		RefSeq:	3118396812C

LocusLink provides links into:

- PubMed
- Ommim
- Refseq
- Homologene
- Unigene
- Variation data

NCBI HomoloGene

Search: LocusLink

Organisms: Human

Organism: Human

Definitions:

Human: Human is a collection of curated and calculated homologs for genes as represented by LocusLink, and their transcripts. By gene, transcript, and calculated contig using whole genome shotgun sequencing.

Curated homologs include orthologous pairs reported in the Mouse Genome Database (MGD) at the Jackson Laboratory, the Comparative Genomics Network (CGN) at the University of Oregon, and in pathway reports.

The calculated homologs are the result of nucleotide sequence comparisons between a pair of organisms. The organisms represented here are the mouse (Mus musculus), rat (Rattus norvegicus), cow (Bos taurus), dog (Canis lupus familiaris), and the platypus (Ornithorhynchus anatinus). Calculated homologs are also available for Chlamydia trachomatis, Neisseria gonorrhoeae, and Chlamydia pneumoniae. A calculated homolog is represented using genomic sequences, while a curated homolog is represented using assembled contigs (contig).

Calculated Homologs:

Alignments [Zhang et al.] to identify unique nucleotide sequences for each pair of organisms, and to identify those sequence pairs that share the highest degree of nucleotide sequence similarity.

The best match for a sequence of one organism to a sequence in a second organism is based on the percent of identity of sequences (PID) on an aligned contig (100 base pairs).

Contigs where more sequences are available in one organism than another may result in several sequences in one organism for the same contig. In a homolog alignment, when two sequences are each other's best match (highest PID match for the sequences' corresponding genes) or are considered as being positive orthologs.

Results are updated regularly as more sequences become available and as UniGene is updated.

The current database for the calculated results are available at our ftp site: <http://www.ncbi.nlm.nih.gov/UniGene/HomoloGene>

References:

A paper is currently in press outlining HomoloGene: <http://www.ncbi.nlm.nih.gov/UniGene/HomoloGene>

HOMOLOGENE ENTRY

Mus musculus: H-2D-associated transcript 2 (H2t2)
LocusLink | MGD | UniGene

POSSIBLE HOMOLOGOUS GENES

B. taurus: ESTs, Highly similar to B35598 MHC class II histocompatibility antigen H-2D-associated protein 2 [infect] - Human [Hsap001]
UniGene

H. sapiens: HLA-D-associated transcript 2 (BAT2)
LocusLink | UniGene

R. norvegicus: ESTs, Highly similar to Bat2; DNA segment, Chr 17, human D6S251E, RHEN1;DNA 311029006 gene [Mus musculus] [Mus musculus]
UniGene

CURATED ORTHOLOGS

Published orthologs as reported in curated databases

Organism-Gene	Homology	Organism-Gene
Mus musculus-Bat2	Homo sapiens-BAT2	
	Map	
	Human	
	-	
	Mouse	
	-	
	Human	
Mus musculus-Bat2	RBD	H. sapiens-BAT2

CALCULATED ORTHOLOGS

Listed below are the nucleotide sequence comparisons used in determining homology. The S. ID column includes hyperlinks to the indicated segments.

Organism-Gene	Sequence ID	% Sequence	Organism-Gene
Mus musculus-NM_000027.9 BF-613381	R.norvegicus	-	-

Resources for Genomic Comparison

- ➲ GLASS-<http://plover.lcs.mit.edu>
- ➲ PipMaker: <http://bio.cse.psu.edu>
- ➲ Rosetta: <http://plover.lcs.mit.edu>(genes)
- ➲ SGP: <http://soft.ice.mpg.de/sgp-1>
- ➲ VISTA: <http://www-gsd.lbl.gov/VISTA>
- ➲ WABA:
<http://www.cse.ucsc.edu/~kent/xenoAli/index.html>

EFFICIENT SEARCHING

☞ Use Wild Cards: #,\$,?,*

☞ Use Boolean Operators

- Not
- And
- Or
- Nor

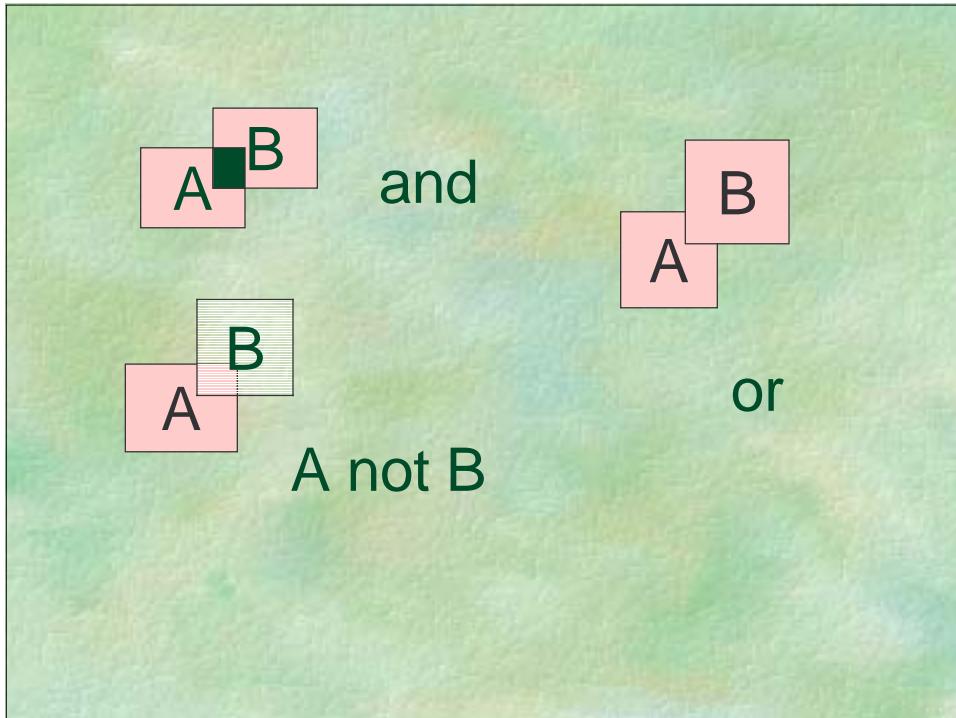
Boolean Operators

☞ AND A and B BOTH

☞ OR A or B EITHER

☞ NOT B not A Have B, do not have A

☞ NOR A nor B A but not B OR B but
not A



WILD CARDS

- Match one character-NCBI uses #
- Match zero or one character NCBI uses \$, others ?
- Match zero or more characters-usually *

RULES OF THUMB

- ☞ Use an up-to-date database; repeat often
- ☞ Choose a fast algorithm
- ☞ Use the most recent version
- ☞ Work at the protein level--for a small amount of evolutionary change, DNA sequence contains less information about homology
- ☞ Respect your own *intuition*

Other Resources

- ☞ NCBI Education Page
<http://www.ncbi.nlm.nih.gov/Education/index.html>
- ☞ BCM Gene Finder
http://searchlauncherbcm.tmc.edu/docs/sl_links.html
- ☞ EBI-SwissProt, TrEMBL, PIR, SRS, Tools
<http://www.ebi.ac.uk>
- ☞ ExPASy-SwissProt, TrEMBL
<http://www.expasy.ch/>
- ☞ DISC-DNA Information and Stock Center
<http://www.dna.affrc.go.jp>

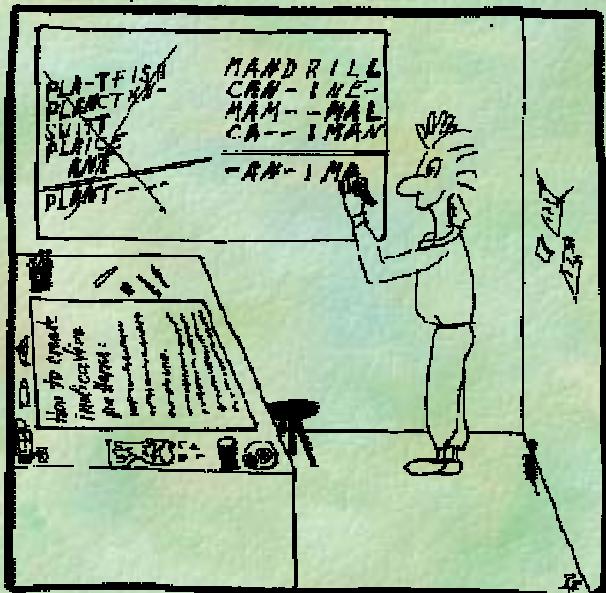
MEDICAL SUBJECT HEADINGS

- ☞ CONTROLLED Vocabulary
- ☞ Indexing of articles, books, etc.
- ☞ Current version has over 300,000 terms
- ☞ Can download list and make your own assortment

MeSH Advantages

- ☞ Assigned to the entire document, not just title and abstract
 - ☞ Major topic (*)
 - ☞ Subheadings if available
 - ☞ MeSH topics are exploded to include all the terms included in the meaning.
- Try it; you may like it.

How we develop Prosite patterns!



Brigitte Boeckmann / 1994

"'Potential', 'Probable', 'By Similarity' –
They don't know anything about this protein!"

Brigitte Boeckmann / 1994

